Aging and Disease
www.aginganddisease.org
Volume 1, Number 3; 199-211, December 2010

Review

The p38 MAP Kinase Family as Regulators of Proinflammatory Cytokine Production in Degenerative Diseases of the CNS

Adam D. Bachstetter [1] and Linda J. Van Eldik [1, 2,*]

[1]Sanders-Brown Center on Aging, [2]Dept Anatomy and Neurobiology, University of Kentucky Lexington, KY 40536-0230 USA

[Received September 7, 2010; Revised September 15, 2010; Accepted September 17, 2010]

ABSTRACT: Inflammation in the central nervous system (CNS) is a common feature of age-related neurodegenerative diseases. Proinflammatory cytokines, such as IL-1β and TNFα, are produced primarily by cells of the innate immune system, namely microglia in the CNS, and are believed to contribute to the neuronal damage seen in the disease. The p38 mitogen-activated protein kinase (MAPK) is one of the kinase pathways that regulate the production of IL-1β and TNFα. Importantly, small molecule inhibitors of the p38 MAPK family have been developed and show efficacy in blocking the production of IL-1β and TNFα. The p38 family consists of at least four isoforms (p38α, β, γ, δ) encoded by separate genes. Recent studies have begun to demonstrate unique functions of the different isoforms, with p38α being implicated as the key isoform involved in CNS inflammation. Interestingly, there is also emerging evidence that two downstream substrates of p38 may have opposing roles, with MK2 being pro-inflammatory and MSK1/2 being antiinflammatory. This review discusses the properties, function and regulation of the p38 MAPK family as it relates to cytokine production in the CNS.

Key words: Protein kinase; Neuroinflammation; Neurodegeneration; Microglia; Signal Transduction

One of the primary classes of inflammatory mediators throughout the body, including the central nervous system (CNS), is cytokines. Cytokines are signaling molecules that act through specific receptors and signal transduction pathways to exert a particular biological response in a target cell. Extensive evidence from both clinical studies and preclinical animal models has implicated overproduction of proinflammatory cytokines as a contributor to pathophysiology progression in chronic neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (for review, see [1]). Several studies have also documented a key role for proinflammatory cytokine overproduction as a potential driving force for pathology progression in acute neurodegenerative disorders such as traumatic brain injury and stroke. Taken in its entirety, the evidence is consistent with the hypothesis that proinflammatory cytokine up-regulation is a comparatively early event in the progression of pathophysiology that is causally linked to synaptic dysfunction, behavior deficits and, in the more extreme case, neuronal death. These data also suggest that strategies to target signaling pathways that lead to cytokine overproduction should be explored in attempts to develop new CNS therapeutics with the potential for disease modification in multiple diseases and clinical presentations.

A key signal transduction pathway involved in the production of proinflammatory cytokines is the p38 mitogen-activated protein kinase (MAPK) pathway (Figure 1). Interestingly, p38 MAPK is also one of the primary signal transduction pathways activated by the cytokines IL-1β and TNFα [2-5]. This raises the

***Correspondence should be addressed to:** Dr. Linda J. Van Eldik. 101 Sanders-Brown Building, 800 S. Limestone, Lexington, KY 40536-0230, USA. *Email:* linda.vaneldik@uky.edu
ISSN: 2152-5250

potential for a specific p38 MAPK-activating stimulus to lead to a state where inflammation begets inflammation. In the AD brain, for example, the initial stimulus could be an increase in the protein amyloid-beta (Aβ), which can activate p38 MAPK and proinflammatory cytokine release from microglia. Excessive proinflammatory cytokine production can then lead to further p38 MAPK activation in microglia or other CNS cell types (e.g., astrocytes and neurons) and a resultant potential feedback loop that exacerbates disease. The p38 MAPK pathway, therefore, appears to be a critical nexus between pathological microglial activation and detrimental inflammation in CNS disorders. If this link can be disrupted selectively, balance may be able to be restored.

The goal of this review is to present an overview of the p38 MAPK family as it relates to CNS proinflammatory cytokine production. We provide a basic introduction to the p38 MAPK family that emphasizes the role of individual p38 MAPK isoforms in the production of proinflammatory cytokines. We also discuss various approaches used to implicate particular p38 MAPK isoforms, especially p38α, in cytokine production. Finally, we discuss the interface of p38 MAPK with two of its downstream substrates in the regulation of proinflammatory responses. The overall purpose is to explore the evidence that p38 MAPK signaling pathways may be viable targets for modulating inflammatory responses in neurological diseases.

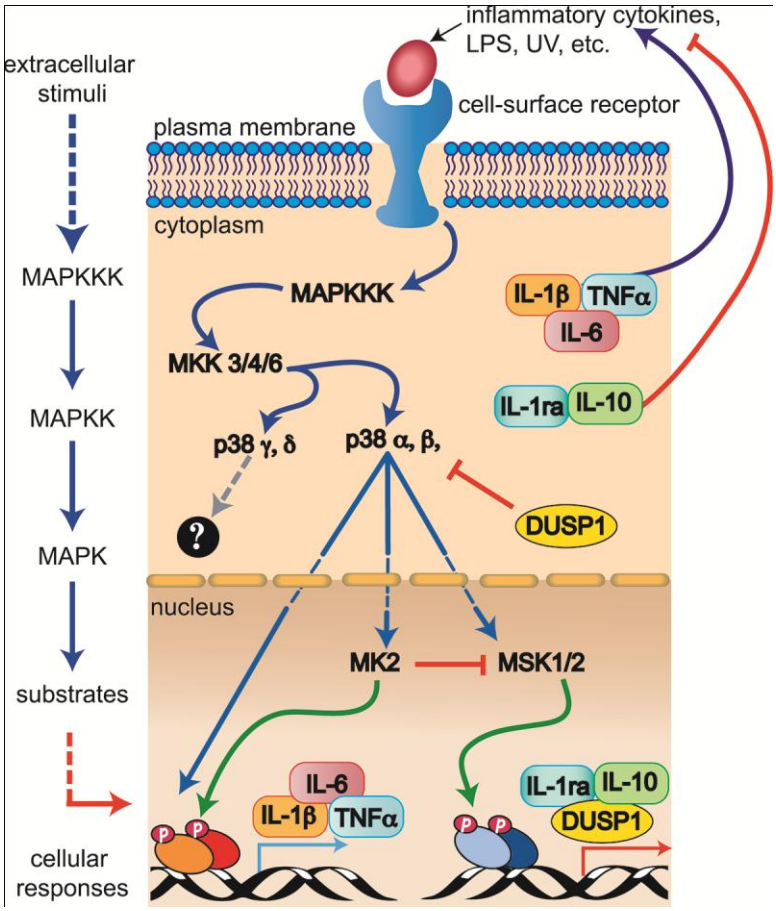

Figure 1: Overview of the p38 MAPK signal transduction pathway involved in cytokine production. Extracelluar stimuli activate receptor and receptor associated proteins, which then transduce the activating signal into cellular responses by a three step MAPK pathway (MAPKKK, MAPKK, MAPK) that leads to p38 activation. Activated p38 can directly activate transcription factors or can act indirectly via two downstream kinases MK2 and MSK1/2. The cellular response produced can have many paracrine functions but can also have autocrine functions leading to further activation or suppression of the p38 pathway.

The p38 family of protein kinases

The serine-threonine protein kinase p38 was identified as a 38kDa protein that is phosphorylated within 15 minutes in response to lipopolysaccharide (LPS) [5, 6]. Subsequent studies demonstrated that p38 (later named p38α) was strongly activated by UV radiation,

and also by the proinflammatory cytokines, IL-1β and TNFα [2-4]. The p38 MAPK family consists of four major isoforms encoded by separate genes: p38α (MAPK14), p38β (MAPK11), p38γ (MAPK12), and p38δ (MAPK13). These isoforms are expressed in different tissues and have distinct functions. In a general sense, the p38 MAPKs integrate and process extracellular stimuli through a series of intracellular signaling complexes and phosphorylation cascades that lead to stimulus-specific responses. In response to inflammatory stimuli, p38 MAPK is activated by a series of upstream kinases that lead to dual phosphorylation of Thr and Tyr residues in p38. The activated p38 MAPK then phosphorylates appropriate downstream substrates that culminate in increased production of proinflammatory cytokines by a number of different mechanisms (reviewed in [7]).

p38α MAPK and p38β MAPK

The α and β isoforms of p38 MAPK share 75% homology [8]. The expression of p38α and p38β is highest in the heart, skeletal muscle and brain, but there are low levels of expression of these two isoforms in most tissues [8, 9]. Most stimuli that activate p38α also activate p38β to a similar degree, although exceptions have been reported. For example, in HeLa cells transfected with either p38α or p38β [8], TNFα and IL-1β both activate p38α. However, TNFα stimulates p38β activity to a much larger degree than does IL-1β. Furthermore, the response kinetics to TNFα are different; p38α is quickly activated within 15 minutes after stimulation, whereas the response by p38β is slower and slightly blunted [8]. The most striking difference was a strong preference by p38β for one of its substrates, ATF2. Overexpression of p38β increased ATF2 reporter gene expression ~20-fold better than p38α [20]. In addition, p38β has ~180-fold higher catalytic activity for ATF2 than does p38α [8,10]. Although the differences between p38α and p38β may be subtle, it is clear that these isoforms may be regulated differently and work via different downstream substrates in response to specific inflammatory stimuli.

Insight into the role of p38α MAPK and p38β MAPK in cytokine responses

A number of different approaches have been used to explore the role of p38 MAPK in inflammatory cytokine production. The pyridinyl imidazole class of compounds was one of the first small molecule inhibitors of p38 MAPK, and have been excellent tools in validating the importance of p38 MAPK in inflammatory cytokine production. However, these early generation p38 MAPK inhibitors were not very selective for p38 and also did not distinguish between p38α and p38β [8], limiting the ability to infer specific functions of the individual p38 isoforms. To further hamper the understanding of the function of the p38α isoform, global deletion of the p38α gene in mice is embryonically lethal, due to a requirement for p38α in placental development and organogenesis [11-14]. On the other hand, mice null for the p38β gene have no known developmental phenotype [15]. Despite these early impediments, newer approaches using drug-resistant knock-in mice, tissue-selective conditional p38α gene deletions, and new classes of more selective inhibitors have provided increased insight into distinct functions of p38α and p38β.

Drug-resistant, knock-in mice

The gatekeeper residue of protein kinases is a key residue in the ATP binding fold whose side chain is situated in front of a conserved hydrophobic pocket region, but is not required for kinase activity. In most protein kinases, the gatekeeper residue is an amino acid with a bulky side chain such as Met, Leu, or Phe that will block access to this pocket [16]. However, in p38α and p38β, the gatekeeper residue is an amino acid with a small side chain (Thr-106), which allows entrance to this hydrophobic region. Many p38 inhibitors utilize the accessibility of the hydrophobic pocket for specificity for the p38α and p38β isoforms over the p38γ/p38δ isoforms which have a larger Met gatekeeper residue. In addition, many inhibitors including the pyridinyl imidazole based inhibitors [17, 18] utilize stabilizing hydrogen bond interactions with the amide nitrogen of Met-109 for increased inhibitor affinity.

O'Keefe et al [17, 18] exploited the kinase gatekeeper selectivity filter to generate a point mutant of either p38α or p38β where Met is substituted for Thr at position 106 (T106M). The mutant kinase retains full kinase activity, but is no longer susceptible to inhibition by small molecule inhibitors that utilize this structural feature. They then generated mouse strains that have either a knocked-in p38α(T106M) or p38β(T106M) whose insertion disrupts the endogenous p38 isoform. These mice retain full p38 kinase activity, but the enzyme can no longer bind to the p38 inhibitors. For the first time the question could be asked *in vivo* which p38 isoform, α or β, was

responsible for the effects of p38 inhibitors. The answer: p38α.

In the p38α (T106M) mice, O'Keefe and colleagues [17,18] demonstrated that it was indeed the p38α form of the kinase that was important for cytokine production. Treatment of either wild type mice or p38β (T106M) mice with a p38 inhibitor was equally effective at blocking LPS-induced increases in TNFα in the plasma. However, in the drug resistant, p38α (T106M) mice, the p38 inhibitor was completely ineffective [18]. This nicely designed study, for the first time, clearly showed that selective inhibition of the p38α isoform *in vivo* is sufficient and necessary for suppression of peripherally increased proinflammatory cytokine levels after an inflammatory challenge. Whether similar results occur in CNS inflammatory conditions has not been reported yet, but these knock-in mice will provide valuable tools for validating that new CNS-penetrant small molecule cytokine suppressors are exerting their CNS effects via inhibition of p38α.

Conditional p38α knock-out mice

The second approach to study of p38α is the use of tissue-selective, conditional knock-out (KO) mice. This approach has been very fruitful in enhancing our understanding of what p38α does in different cell types. In 2008, Kang and colleagues [19] developed a mouse that was null for p38α only in cells of the monocyte/macrophage lineage (p38αΔM). Following either LPS stimulation or cecal ligation and puncture, p38αΔM mice were protected from the lethal effects of both LPS and cecal ligation and puncture induced sepsis. The protective effect appeared to be due to a reduced TNFα response, which in both sepsis models was almost absent in the p38αΔM mice [19]. Another *in vivo* model that has been tested in the p38αΔM mice is a skin inflammation model. This tissue injury model produced some unexpected findings. Despite the protection that had been shown against septic shock, the p38αΔM mice had a worse outcome in the skin inflammation model. There are many possible explanations for why in this model the injury was exacerbated, but two responses that may have contributed were an increase in COX-2 expression and a decrease in the anti-inflammatory cytokine IL-10 in the p38αΔM mice compared to wild type mice [20].

Kim et al. [20] took the study of the p38αΔM mice a step further, to implicate another potential mechanism for the increased inflammation. There are multiple points in the p38 MAPK signaling pathways where regulation can occur. Points of negative regulation include receptor desensitization, dissociation or deactivation of signaling pathway mediators, and kinase dephosphorylation by phosphatases. Dephosphorylation of some protein kinases is a fast and efficient way of shutting down kinase activity [21]. One phosphatase that is important for dephosphorylation of p38 MAPK is dual-specificity protein phosphatase (DUSP)1 (also known as MKP-1). DUSP1 is an immediate-early gene which is involved in the dephosphorylation of p38 and JNK, and also to a lesser extent ERK [21]. Macrophages isolated from DUSP1 deficient mice have sustained activation of p38, and increased TNF-α following LPS [22]. The temporal activation of DUSP1 occurs shortly after that of p38. For example, following LPS stimulation, DUSP1 protein becomes elevated at 45 minutes and peaks at 60 minutes. In contrast, phosho-p38 expression peaks at 15 minutes and reaches baseline at 45 minutes [22]. In DUSP1 deficient macrophages, LPS-induced activation and subsequent deactivation of phospho-p38 were significantly slowed, resulting in prolonged duration of action [23]. Importantly, a dose of LPS that was normally sub-lethal in wild type mice was 80% lethal in DUSP1 deficient mice by 72 hours [23], demonstrating that DUSP1 plays a role in limiting p38 activation.

An interesting observation is that p38α activation and signaling can also drive DUSP1 production, thus providing a mechanism for negative feedback to limit p38 activation [20]. The p38α null macrophages showed a significantly diminished induction of DUSP1 in response to LPS. In addition, as DUSP1 also dephosphorylates JNK and ERK, the decrease in DUSP1 seen in the p38αΔM resulted in an increase in phospho-JNK and phospho-ERK. Thus, the increased inflammation seen in the p38αΔM may result from both inadequate DUSP1 production as well as increases in other MAPK pathways (JNK and ERK) that may converge on inflammatory responses.

p38α-selective small molecule inhibitors

The third approach to study the function of p38α is through the use of newer, more isoform selective p38 inhibitors that have been designed using the extensive crystallographic data available on p38 structures. This is also the only approach of the three that has been used to explore the role of p38α in brain injury and neurodegenerative disease, and there are two examples of the effectiveness of the p38α specific

inhibitors in preventing neurodegeneration. The first example was shown using the second-generation p38α inhibitor, SB239063, in the middle cerebral artery occlusion (MCAO) model of brain injury. There was a reduction in the infarct volume following MCAO in rats that received the small molecule p38α inhibitor, as well as a lessening of the neurological deficits following injury [24, 25].

A second example implicating p38α in CNS disorders was demonstrated in an AD-relevant mouse model. A selective, CNS-penetrant p38α inhibitor, MW01-2-069A-SRM, was developed and used by Munoz et al. [26] in an amyloid-beta (Aβ)-induced model of brain injury. MW01-2069A-SRM was found to block the Aβ-induced increase in levels of the proinflammatory cytokines TNFα and IL-1β in the hippocampus. The p38α inhibitor also prevented synaptic dysfunction and hippocampal-dependent behavioral deficits caused by the Aβ insult [26]. These studies indicate that p38α could be a credible target for drug development campaigns for AD and related neurodegenerative diseases where overproduction of cytokines is involved in the disease mechanisms.

A recent study highlighted a potential caveat to the use of non-selective p38α/p38β inhibitors. Greenblatt et al. [27] found that in p38β null mice, which previously have been reported to have no phenotype, there were significant skeletal abnormalities including a thinning of the bones. There was also some thinning in the p38α conditional KO mouse, but the effect was less pronounced. The thinning of the bones was also seen after treatment with the non-selective p38α/p38β inhibitor SB203580 [27]. The authors suggest that p38β agonists may help prevent osteoporosis. Development of selective p38α inhibitors that spare p38β might need to be considered for an aging population where osteoporosis can be a complication.

p38γ MAPK and p38δ MAPK

The highest expression of p38δ is limited to the lungs and kidneys [28]; yet, in a very intriguing study, Hale et al. [29] investigated the expression of the four p38 MAPK isoforms in different immune cells. The p38α isoform was widely expressed, and was the dominant p38 isoform in the immune cell study. Endothelial cells were the one exception. In endothelial cells, lower levels of p38α were found and there was robust expression of p38β. The interesting finding concerning p38δ resulted from a comparison of monocytes and macrophages. In monocytes, the precursor to dendritic cells and macrophages, there were high levels of p38α and only low levels of p38δ. However, in monocytes that had been differentiated into macrophages *in vitro,* the levels of p38α were reduced compared to the levels in monocytes. As determined by real-time PCR, in this population of macrophages, p38δ became the dominant p38 isoform, with ~4-fold increased expression compared to p38α [29].

Fearns et al. [30] demonstrated using monocyte cell lines and an astrocytoma cell line that IL-1β and TNFα, or TLR-2 or TLR-4 ligands could activate all four p38 family members. The degree of activation of the different p38 family members was cell and stimulus specific. For example, in the astrocytoma cell line, IL-1β caused the greatest induction in p38β, p38γ, and p38δ, whereas LPS caused the greatest increase in p38α. In the THP1- wtCD14 monocyte cell line, the TLR-2 ligand caused a large induction of all 4 isoforms, whereas the cytokines activated p38α, but not p38β, p38γ, or p38δ [30]. The expression of p38δ has not been looked at in microglia. It is also not known if there are changes in the expression of p38δ in neurodegenerative disease.

The p38δ (MAPK13) isoform shares 60% homology with the other p38 family members [28], and can be acted on by the upstream MAPKKK and MAPKK (MKK3, MKK4, and MKK6) kinases that activate other p38 family members [28]. This suggests that p38δ should be activated by stimuli that activate the other p38 family members. This was confirmed by demonstrating activation of p38δ kinase activity after stimulation by anisomycin, UV radiation and TNFα that was similar to that of p38α kinase activation. However, IL-1β and IL-6 were found to be weaker activators of p38δ compared to p38α [28]. In response to LPS, macrophages exhibited an increase in a 41kDa phospho-p38 band which represented phospho-p38α, and a 43.5kDa band which represented phospho-p38δ [29]. This separation into phospho-p38α and phospho-p38δ could also be seen in PC12 cells following hypoxia [31]. In COS-7 cells transfected with tau and each of the four p38 isoforms, it was found that only p38γ and p38δ could efficiently phosphorylate tau [32]. As p38γ and p38δ are not the major p38 isoform in neurons, it is unclear how relevant these results are to Alzheimer's disease. In a subsequent study [33], it was found that all four p38 isoforms could phosphorylate tau, that Thr50 in tau was an important site of phosphorylation by p38, and that phosphorylation of tau at this residue was most

dependent on p38δ. Finally, the authors showed that phosphorylation of Thr50 was found in Alzheimer's disease brains [33].

The final member of the family, p38γ (MAPK12), shares 60% homology with p38 α and β [9]. The expression of p38γ is localized to skeletal muscle. Inflammatory p38 substrates, MK2 and ATF2, are not targets of p38γ [9] and p38γ is not thought to be important in inflammation.

MK2: Is this downstream target of p38 the next logical step in anti-cytokine therapeutics?

Mitogen-activated protein kinase-activated protein kinase 2 (MK2) is one of several kinases activated through direct phosphorylation by p38 [34]. MK2 kinase activity is dependent on activation by p38α. In p38α negative embryonic stem cells (ES) cells treated with strong p38 activators, anisomycin (50 ng/ml) or sodium arsenite (0.5 mM), there was essentially no MK2 kinase activity [12]. The expression of p38 and MK2 are each reliant on the other, as loss of MK2 causes a decrease in the amount of total p38 [35].

In 1999, Kotlyarov and colleagues [36] developed a MK2 KO mouse and established the importance of MK2 in regulating TNFα. Upon high dose LPS stimulation, which was lethal in wild type mice, the MK2 KO mice were found to be resistant to the LPS injury. The reasons for the resistance were that there was only a very limited cytokine storm in response to the LPS, and there was a decreased induction of a number of cytokines including IFN-γ, IL-β IL-6, and IL-10. In addition, there was essentially no up-regulation of TNFα in the serum of these mice, as well as a dramatic decrease in nitric oxide levels [36]. MAP kinase-activated protein kinase 3 (MK3) is 70% identical in structure to MK2. Lack of MK3 alone does not produce an altered cytokine response; however, the absence of both MK2 and MK3 does produce a further decrease in TNFα from the levels of the MK2 KO alone [37]. MK2 was subsequently shown to directly regulate TNFα and IL-6 at the 3'-untranslated region (UTR) of mRNA in the AU-rich elements (AREs) and thereby regulate mRNA stability [38]. Interestingly, MK2 also inhibits the phosphorylation of MSK1/2 by p38 [39] (See figure 1 for simplified overview of interactions). In the CNS, the expression of MK2 can be found in neurons, astrocytes, and microglia; however, like p38α, the expression of MK2 in microglia is 5 to 10 fold higher than in neurons or astrocytes [40]. In microglia stimulated with LPS and IFN-γ, 75% of the increase in TNFα can be blocked by MK2 genetic deletion [40]. A reduction in TNFα, IL-6, KC and nitric oxide was found in mixed neuron-glia cultures treated with LPS from MK2 KO mice compared to wild type mice [41].

The effects of MK2 deficiency have been investigated in a number of neurological disease models, including stroke, AD, and PD. MK2 KO mice were protected from stroke in either a permanent or transient MCAO model, and the loss of MK2 almost completely prevented the up-regulation of IL-1β that was seen in the wild type mice [42]. In neuron-glia co-cultures treated with Aβ, there was decreased Aβ-induced neuronal death when the microglia were from MK2 KO mice [40]. In the MPTP mouse model of Parkinson's disease, there was a sparing of the tyrosine hydroxylase positive neurons in the MK2 KO mice [41]. While there was a decrease in TNFα and nitric oxide expression in the MK2 KO mice following MPTP, there was no change in the microglia activation as measured by MAC-1 expression [41]. Decreased TNFα responses in Parkinson's disease models have previously been shown to be neuroprotective [43,44]. Therefore, it is likely that the reduced TNFα response in the MK2 KO mice correlates with the neuroprotective effect in the MPTP model [41].

To explore potential mechanisms by which MK2 mice neurons were protected from the MPTP, Thomas et al. [41] used neuron-glia co-culture. Suspecting that the cell death was mediated via nitric oxide, they tested to see if loss of MK2 in the neurons was directly protecting neurons from nitric oxide-induced cell death. Using a chemical nitric oxide donor, 100μM sodium nitroprusside, nitric oxide-induced cell death in enriched neuronal cultures was found to be independent of MK2. Interestingly, pretreatment of the neuronal cultures with a p38 inhibitor did protect the neurons from the nitric oxide. The neuronal protection by p38 inhibition was similar for both the MK2+/+ and MK2-/- neurons. Thus, the neuronal survival effects of p38 inhibition in response to nitric oxide are a result of an MK2 independent mechanism [41].

Therapeutically it appears that there may be different conditions when targeting only MK2 might be useful, in comparison to times when a p38 inhibitor may be indicated. For example, it is clear that many of the anti-cytokine effects of blocking p38 are a result of inhibition of MK2; therefore if the goal were to

block cytokine up-regulation following a traumatic brain injury, for example, an MK2 inhibitor could be useful. However, the MK2 inhibitor would appear to lack some of the direct neuroprotective effects of a p38 inhibitor. Depending on the clinical indication then, it may be advantageous to use a p38 inhibitor that will have an impact on both neurons and glia, or a more specific microglia cytokine suppressor with an MK2 inhibitor.

A complicating factor that must be kept in mind when targeting intracellular kinases is that phosphorylation signaling pathways have considerable crosstalk. There are interconnected loops that contribute both positive and negative feedback and others that modulate the response. For example, it has been reported [39] that MK2 can modulate the NFκB response, causing a sustained activation of NFκB by maintaining NFκB in the nucleus. In the absence of MK2, NFκB is quickly shut off; however the initial NFκB response is stronger. The regulation of NFκB by p38 occurs by the ability of the p38 and NFκB to move in and out of the nucleus [39]. Thus, there is a complex and interconnected system of cross-talking pathways potentially regulated by p38 levels.

The first p38 inhibitors, the pyridinyl imidazole class of compounds, were described in 1994 [45]. It wasn't until 2005, however, when the first small molecule inhibitor of MK2, an aminocyanopyridine, was reported [46]. Since 2005, a number of MK2 inhibitors have been developed. The carboline analogs class of MK2 inhibitors, developed at Boehringer-Ingelheim Pharmaceuticals, have been shown by crystal structure to interact with the hinge region of MK2 (M138–D142) and bind between p-loop and amino acids V118, T206, L193, and Y194. Unfortunately, the first group of carboline analogs was reported to lack activity in cell based assays [47]. In 2008, a pyrazinoindolone class of MK2 inhibitors was described [48]. The lead compound had a reported IC50 for MK2 kinase activity of 3nM and a cell based IC50 for TNFα at 300nM, but the bioavailability of the compound was not sufficient for TNFα inhibition *in vivo* [48]. However, a number of new MK2 inhibitors have been shown to have the ability to inhibit TNFα *in vivo*. For example, Velcicky et at. [49] modified the pyrrolo-pyrimidinone scaffold to synthesize a 3aminopyrazole based inhibitor. At a dose of 100 mg/kg given orally, the aminopyrazole compound caused a 68% reduction in serum levels of TNFα [49]. Another MK2 inhibitor, a β-carboline derivative, was synthesized as an ester prodrug [50] and resulted in an 84% inhibition of TNFα when administered at 40mg/kg IP in a rat LPS model.

So far, the MK2 inhibitors are still in the development phase. There is only limited efficacy of a small subset of the inhibitors using *in vivo* models. As of the writing of this review, there have been no tests of MK2 inhibitors in disease relevant models such as rheumatoid arthritis. There have been no reported studies in CNS disorders or even information available on whether the compounds are brain-penetrant. Therefore, although MK2 would appear to be a good therapeutic target for CNS disorders, more work is needed. In the last five years, much progress has been made in identifying novel small molecules that have the ability to inhibit MK2. Effort must be expended now to refine these compounds to improve their bioavailability and CNS potential so that they can be explored for their utility in inflammatory CNS disorders (for a recent review on the topic see [51]).

MSK-1/2: Is this downstream target of p38 the brake on runaway inflammation?

Mitogen- and stress-activated kinases 1 and 2 (MSK1 and 2) are two related kinases that are activated downstream of p38 and Erk1/2 [52]. Both MSK1 and MSK2 are important for CREB and ATF1 phosphorylation in response to stress (UV or anisomycin) but not in response to mitogen (epidermal growth factor) [53]. MSK-1 can act on NFκB to cause prolonged activation [39]. The single deletion of either MSK isoform caused only a small reduction in the cellular response to either stress-induced or mitogen-induced stimuli [53]. However, in the absence of both MSK1 and MSK2, there was a dramatic reduction in phosphorylation of CREB and ATF1. Two CREB-dependent immediate early genes c-fos and junB also showed a blunted induction in response to anisomycin (a strong p38 activator) in the MSK1/2 KO fibroblasts, but not in the single MSK deletion [53].

As a downstream target of p38, MSK1/2 was postulated to be important for the production of proinflammatory cytokines, reminiscent of the role of MK2. This question was addressed directly by Ananieva et al. [20, 54], where they found that MSK1/2 has anti-inflammatory activities and helps restrain inflammation. Using the MSK1/2 double KO mice, they found that there was a dramatic 20 to 60-fold increase in TNFα, IL-6 and IL-12 in bone marrow derived macrophages stimulated with LPS. Compared

to the WT bone marrow derived macrophages, there was also a decrease in the anti-inflammatory cytokine IL-10 in the MSK1/2 double KO macrophages [20, 54]. Using cells obtained from a triple KO mouse for MSK1/2 and IL-10, Ananieva et al. [20, 54] showed that the increase in TNF and IL-12 was directly dependent on MSK1/2. The triple KO mouse macrophages had greater IL-12 and TNF than macrophages from the single IL-10 KO. However, the increase in IL-6 was dependent on IL-10, as both the triple KO and the single IL-10 KO had similar levels of IL-6. When tested *in vivo*, a similar increase in TNF, IL-6, IL-12 and a decrease in IL-10 were found in the serum of mice injected with LPS [54]. In addition to IL-10, MSK1/2 is also important for the production of IL-1 receptor antagonist (IL-1ra) in response to LPS, partially independent of IL-10 [55]. Finally, MSK1/2 was also shown to increase DUSP1 transcription in macrophages, which can promote p38 dephosphorylation and deactivation [20, 54].

While the role of MSK1/2 has not been tested in any CNS-relevant *in vivo* models, it has been shown that MSK1 is expressed in the CNS. Webber et al. [56] measured MSK1 phosphorylation in the hippocampus and cortex of 10 AD and 9 non-AD patients, and found that phospho-MSK1 was dramatically increased in the cytoplasm of the pyramidal neurons in the AD brain. This was true for MSK-1 phosphorylated at Thr581 and MSK1 phosphorylated at Ser376, although the staining patterns for the two phospho-specific antibodies did not overlap. In the non-AD samples, there appeared to be no phospho-MSK-1 staining. Interestingly, the pattern of MSK-1 staining co-localized with phospho-ERK and not with phospho-p38. One caveat is that the staining was done on adjacent sections and not using confocal microscopy, so it is difficult to determine how much colocalization occurred in these samples [56].

By gene deletion studies, the p38 substrate MSK1/2 has clearly been shown to have anti-inflammatory properties (Fig.1). However, almost two decades of research have shown that p38 inhibitors are able to suppress the production of pro-inflammatory cytokines. One possible explanation for these seemingly contradictory findings could come from the way MSK1/2 suppresses inflammation, which can be by acting on p38 (Fig.2). Therefore if p38 is being blocked with a compound then suppression of MSK1/2 has a negated impact. The exception would be the decrease in IL-10, which suppresses inflammation via a Jak/Stat mediated mechanism (for a recent review see [57]). Pharmacological inhibition typically does not completely inhibit the biological target the way a gene deletion does, which must be considered in interpreting data obtained from these two kinds of studies. In addition, in considering p38-driven signaling pathways as potential therapeutic targets, these studies provide another example of the importance of the therapeutic window. It will be important in the future to determine the appropriate window of responsiveness where there is adequate suppression of the pro-inflammatory responses while maintaining some of the anti-inflammatory responsiveness as well.

Is p38 MAPK pathway a potential therapeutic target for CNS inflammatory disorders?

The p38α MAPK is one of the most well established therapeutic targets for peripheral inflammatory diseases, and several small molecule p38α inhibitors are currently in human clinical trials for diseases such as rheumatoid arthritis, asthma, atherosclerosis, and acute lung injury [58]. There has also been a move in recent years to using other members of the MAPK pathway as therapeutic targets, such as MK2 or DUSPs (for recent review see [59, 60]). As we discuss in this review, there is extensive evidence that the p38 MAPK signaling cascade contributes to the cytokine overproduction in disease. Cytokine overproduction contributes to the neurodegenerative sequelae seen in a variety of neurodegenerative disorders. In considering whether a protein of interest should be explored as a potential therapeutic target for CNS inflammatory disorders, two important questions must be addressed. First, is the target present in the right time and place; i.e., is there evidence that it is involved in the disease process? Second, and more importantly, is the target a "druggable target"? In other words, can the target be modulated with a small molecule compound and achieve a therapeutically relevant outcome without untoward adverse effects? Examination of the available literature suggests that p38α fulfills these two criteria for several CNS disorders.

Highlights from the AD field are presented that exemplify the evidence. To the first question – ***right time and place***? For p38α there is extensive evidence to address this question in AD. **Right time:** Stimulation of glial cell cultures with Aβ1-42 induces p38 MAPK activation and subsequent induction of

proinflammatory cytokines [61-66]. Activation of neuronal p38 MAPK by RAGE contributes to translocation of Aβ into neurons, Aβ-induced impairment of cortical LTP, deficits in kinesin-dependent fast axonal transport, and Aβ-induced neurotoxicity [67-70]. The p38 MAPK can phosphorylate tau, *in vitro*, at AD-relevant amino acid residues [71-75], and is involved in cytokine-induced changes in tau phosphorylation and synaptic damage [76]. **Right place:** The p38 MAPK pathway is activated in the early stages of patients with AD and localizes to both neurons and glia [72,73,77-81]. Increases in activated p38 MAPK are seen coincident with AD-relevant pathology endpoints in animal models, such as increases in amyloid deposition, tau phosphorylation and loss of synaptophysin [64-66]. To the second question – ***is it a druggable target?*** A selective, orally active, brain-penetrant p38α inhibitor restores excessive glia proinflammatory cytokine production back towards normal and attenuates neurologic dysfunction in an AD-relevant mouse model, without observable adverse effects [26]. Altogether, p38α MAPK is emerging as an attractive target for CNS inflammatory disorders. Current drug discovery efforts are building on this mechanistic knowledge in attempts to develop potential neurodegenerative disease-modifying therapeutics that target this critical gene-regulating protein kinase. Less evidence is currently available for MK2. As a direct downstream target of p38α, MK2 would appear to be a logical suspect to investigate. It is still open to debate if MK2 is a good therapeutic target for CNS inflammatory disorders, but the odds are in favor of MK2 becoming an attractive avenue of research in the future.

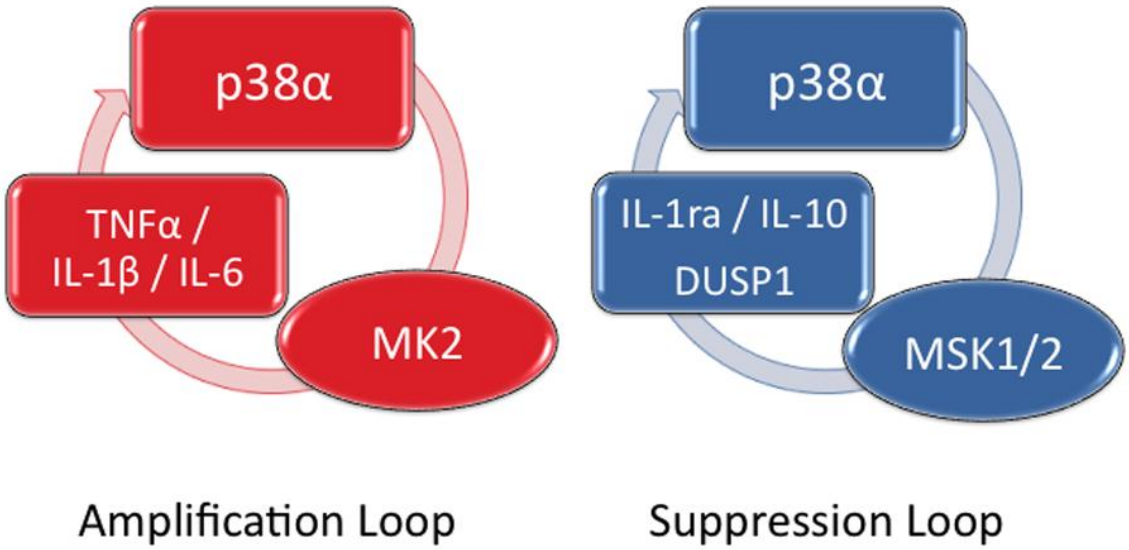

Figure 2: Feedback mechanisms regulating p38α. Inflammatory signals, such as TNFα and IL-1β, can activate the p38 pathway. Two downstream kinases of p38 are MK2 and MSK1/2. MK2 can promote an increase in TNFα, IL-1β and IL-6. These pro-inflammatory cytokines can then feed back onto the cell, activating the same pathway and amplifying the inflammation. MSK1/2 produces the phosphatase DUSP1, which dephosphorylates and deactivates p38. Two antiinflammatory cytokines IL-1ra and IL-10 are also regulated by MSK1/2 activation. These cytokines have the potential to antagonize the effects of IL-1β and suppress the inflammatory response.

Conclusion

Activation of the innate immune system occurs following pathogen invasion, chemical irritation, and mechanical trauma. The acute inflammatory response limits ongoing infection and compartmentalizes damaged tissue. Failure to limit excessive activation of the inflammatory response produces acute disease such as septic shock, which can be fatal. Failure to temporally limit the

inflammatory response produces chronic disease, such as arthritis, asthma, atherosclerosis and age-related neurodegenerative diseases. Many kinases are involved in the inflammatory response. This review has focused on the role of the p38 MAPK family of kinases and the downstream kinases MK2 and MSK1/2 in cytokine production. Figure 1 shows a very simplified flow diagram of p38α signaling, with figure 2 showing how p38α through MK2 and MSK1/2 can affect cytokine production.

Although there has been extensive effort to understand and exploit the p38 MAPK pathway as therapeutic targets for peripheral inflammatory disorders, the field is just beginning to understand how to effectively use our cumulative knowledge to develop strategies to intervene in CNS inflammatory disorders. Selective, CNS-penetrant, small molecule inhibitors of p38α, which have the potential to be used to treat disorders of the CNS, are being developed. One example of a promising, selective p38α inhibitor that has the ability to cross the blood-brain barrier is MW01-2069A-SRM [26]. Small molecule inhibitors of MK2 are still in the early phases of development, and their potential use in CNS disorders needs to be explored. The pivotal role of p38 MAPK pathways in the regulation of responses that lead to overproduction of CNS proinflammatory cytokines emphasizes the importance of these drug discovery efforts.

Acknowledgements

The authors acknowledge funding from the National Institutes of Health (R01 NS064247), the American Health Assistance Foundation (A2008-022), and the Alzheimer's Association (Zenith Award ZEN-09-134506). ADB is supported by NIH fellowship F32 AG037280.

References

[1] Van Eldik, L.J., Thompson, W.L., Ralay Ranaivo, H., Behanna, H.A. and Watterson, D.M. (2007). Glia proinflammatory cytokine upregulation as a therapeutic target for neurodegenerative diseases: function-based and target-based discovery approaches. Int Rev Neurobiol, 82, 277-96.

[2] Raingeaud, J., Gupta, S., Rogers, J.S., Dickens, M., Han, J., Ulevitch, R.J. and Davis, R.J. (1995). Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. J Biol Chem, 270, 7420-6.

[3] Rouse, J., Cohen, P., Trigon, S., Morange, M., Alonso-Llamazares, A., Zamanillo, D., Hunt, and Nebreda, A.R. (1994). A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. Cell, 78, 1027-37.

[4] Freshney, N.W., Rawlinson, L., Guesdon, F., Jones, E., Cowley, S., Hsuan, J. and Saklatvala, J. (1994). Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27. Cell, 78, 1039-49.

[5] Han, J., Lee, J.D., Bibbs, L. and Ulevitch, R.J. (1994). A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science, 265, 808-11.

[6] Han, J., Lee, J.D., Tobias, P.S. and Ulevitch, R.J. (1993). Endotoxin induces rapid protein tyrosine phosphorylation in 70Z/3 cells expressing CD14. J Biol Chem, 268, 25009-14.

[7] Schieven, G.L. (2009). The p38alpha kinase plays a central role in inflammation. Curr Top Med Chem, 9, 1038-48.

[8] Jiang, Y., Chen, C., Li, Z., Guo, W., Gegner, J.A., Lin, S. and Han, J. (1996). Characterization of the structure and function of a new mitogen-activated protein kinase (p38beta). J Biol Chem 271, 17920-6.

[9] Li, Z., Jiang, Y., Ulevitch, R.J. and Han, J. (1996). The primary structure of p38 gamma: a new member of p38 group of MAP kinases. Biochem Biophys Res Commun, 228, 334-40.

[10] Stein, B., Yang, M.X., Young, D.B., Janknecht, R., Hunter, T., Murray, B.W. and Barbosa, M.S. (1997). p38-2, a novel mitogen-activated protein kinase with distinct properties. J Biol Chem, 272, 19509-17.

[11] Tamura, K., Sudo, T., Senftleben, U., Dadak, A.M., Johnson, R. and Karin, M. (2000). Requirement for p38alpha in erythropoietin expression: a role for stress kinases in erythropoiesis. Cell, 102, 221-31.

[12] Allen, M., Svensson, L., Roach, M., Hambor, J., McNeish, J. and Gabel, C.A. (2000). Deficiency of the stress kinase p38alpha results in embryonic lethality: characterization of the kinase dependence of stress responses of enzyme-deficient embryonic stem cells. J Exp Med, 191, 859-70.

[13] Mudgett, J.S., Ding, J., Guh-Siesel, L., Chartrain, N.A., Yang, L., Gopal, S. and Shen, M.M. (2000). Essential role for p38alpha mitogen-activated protein kinase in placental angiogenesis. Proc Natl Acad Sci U S A, 97, 10454-9.

[14] Adams, R.H. et al. (2000). Essential role of p38alpha MAP kinase in placental but not embryonic cardiovascular development. Mol Cell, 6, 109-16.

[15] Beardmore, V.A. et al. (2005). Generation and characterization of p38beta (MAPK11) gene-targeted mice. Mol Cell Biol, 25, 10454-64.

[16] Zhang, C., Kenski, D.M., Paulson, J.L., Bonshtien, A., Sessa, G., Cross, J.V., Templeton, D.J. and Shokat, K.M. (2005). A second-site suppressor strategy for chemical genetic analysis of diverse protein kinases. Nat Methods, 2, 435-41.

[17] Lisnock, J. et al. (1998). Molecular basis for p38 protein kinase inhibitor specificity. Biochemistry, 37, 16573-81.

[18] O'Keefe, S.J. et al. (2007). Chemical genetics define the roles of p38alpha and p38beta in acute and chronic inflammation. J Biol Chem 282, 34663-71.

[19] Kang, Y.J., Chen, J., Otsuka, M., Mols, J., Ren, S., Wang, Y. and Han, J. (2008). Macrophage deletion of p38alpha partially impairs lipopolysaccharide-induced cellular activation. J Immunol, 180, 5075-82.

[20] Kim, C. et al. (2008). The kinase p38 alpha serves cell type-specific inflammatory functions in skin injury and coordinates pro- and anti-inflammatory gene expression. Nat Immunol, 9, 1019-27.

[21] Liu, Y., Shepherd, E.G. and Nelin, L.D. (2007). MAPK phosphatases--regulating the immune response. Nat Rev Immunol, 7, 202-12.

[22] Zhao, Q., Shepherd, E.G., Manson, M.E., Nelin, L.D., Sorokin, A. and Liu, Y. (2005). The role of mitogen-activated protein kinase phosphatase-1 in the response of alveolar macrophages to lipopolysaccharide: attenuation of proinflammatory cytokine biosynthesis via feedback control of p38. J Biol Chem, 280, 8101-8.

[23] Zhao, Q. et al. (2006). MAP kinase phosphatase 1 controls innate immune responses and suppresses endotoxic shock. J Exp Med, 203, 131-40.

[24] Legos, J.J., Erhardt, J.A., White, R.F., Lenhard, S.C., Chandra, S., Parsons, A.A., Tuma, R.F. and Barone, F.C. (2001). SB 239063, a novel p38 inhibitor, attenuates early neuronal injury following ischemia. Brain Res, 892, 70-7.

[25] Barone, F.C. et al. (2001). SB 239063, a second-generation p38 mitogen-activated protein kinase inhibitor, reduces brain injury and neurological deficits in cerebral focal ischemia. J Pharmacol Exp Ther, 296, 312-21.

[26] Munoz, L. et al. (2007). A novel p38 alpha MAPK inhibitor suppresses brain proinflammatory cytokine up-regulation and attenuates synaptic dysfunction and behavioral deficits in an Alzheimer's disease mouse model. J Neuroinflammation, 4, 21.

[27] Greenblatt, M.B. et al. The p38 MAPK pathway is essential for skeletogenesis and bone homeostasis in mice. J Clin Invest, 120, 2457-73.

[28] Jiang, Y. et al. (1997). Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38delta. J Biol Chem, 272, 30122-8.

[29] Hale, K.K., Trollinger, D., Rihanek, M. and Manthey, C.L. (1999). Differential expression and activation of p38 mitogen-activated protein kinase alpha, beta, gamma, and delta in inflammatory cell lineages. J Immunol, 162, 4246-52.

[30] Fearns, C., Kline, L., Gram, H., Di Padova, F., Zurini, M., Han, J. and Ulevitch, R.J. (2000). Coordinate activation of endogenous p38alpha, beta, gamma, and delta by inflammatory stimuli. J Leukoc Biol, 67, 705-11.

[31] Conrad, P.W., Rust, R.T., Han, J., Millhorn, D.E. and Beitner-Johnson, D. (1999). Selective activation of p38alpha and p38gamma by hypoxia. Role in regulation of cyclin D1 by hypoxia in PC12 cells. J Biol Chem, 274, 23570-6.

[32] Buee-Scherrer, V. and Goedert, M. (2002). Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases in intact cells. FEBS Lett, 515, 151-4.

[33] Feijoo, C., Campbell, D.G., Jakes, R., Goedert, M. and Cuenda, A. (2005). Evidence that phosphorylation of the microtubule-associated protein Tau by SAPK4/p38delta at Thr50 promotes microtubule assembly. J Cell Sci, 118, 397-408.

[34] Gaestel, M. (2006). MAPKAP kinases - MKs - two's company, three's a crowd. Nat Rev Mol Cell Biol, 7, 120-30.

[35] Kotlyarov, A., Yannoni, Y., Fritz, S., Laass, K., Telliez, J.B., Pitman, D., Lin, L.L. and Gaestel, M. (2002). Distinct cellular functions of MK2. Mol Cell Biol, 22, 4827-35.

[36] Kotlyarov, A., Neininger, A., Schubert, C., Eckert, R., Birchmeier, C., Volk, H.D. and Gaestel, M. (1999). MAPKAP kinase 2 is essential for LPS-induced TNF-alpha biosynthesis. Nat Cell Biol, 1, 94-7.

[37] Ronkina, N. et al. (2007). The mitogen-activated protein kinase (MAPK)-activated protein kinases MK2 and MK3 cooperate in stimulation of tumor necrosis factor biosynthesis and stabilization of p38 MAPK. Mol Cell Biol, 27, 170-81.

[38] Neininger, A. et al. (2002). MK2 targets AU-rich elements and regulates biosynthesis of tumor necrosis factor and interleukin-6 independently at different post-transcriptional levels. J Biol Chem, 277, 3065-8.

[39] Gorska, M.M. et al. (2007). MK2 controls the level of negative feedback in the NF-kappaB pathway and is essential for vascular permeability and airway inflammation. J Exp Med, 204, 1637-52.

[40] Culbert, A.A. et al. (2006). MAPK-activated protein kinase 2 deficiency in microglia inhibits pro-inflammatory mediator release and resultant neurotoxicity. Relevance to neuroinflammation in a transgenic mouse model of Alzheimer disease. J Biol Chem, 281, 23658-67.

[41] Thomas, T., Timmer, M., Cesnulevicius, K., Hitti, E., Kotlyarov, A. and Gaestel, M. (2008). MAPKAP kinase 2-deficiency prevents neurons from cell death by reducing neuroinflammation-relevance in a mouse model of Parkinson's disease. J Neurochem, 105, 2039-52.

[42] Wang, X., Xu, L., Wang, H., Young, P.R., Gaestel, M. and Feuerstein, G.Z. (2002). Mitogen-activated protein kinase-activated protein (MAPKAP) kinase 2 deficiency protects brain from ischemic injury in mice. J Biol Chem, 277, 43968-72.

[43] Ferger, B., Leng, A., Mura, A., Hengerer, B. and Feldon, J. (2004). Genetic ablation of tumor necrosis factor-alpha (TNF-alpha) and pharmacological inhibition of TNF-synthesis attenuates MPTP toxicity in mouse striatum. J Neurochem, 89, 822-33.

[44] Gemma, C. et al. (2007). Early inhibition of TNFalpha increases 6-hydroxydopamineinduced striatal degeneration. Brain Res 1147, 240-7.

[45] Lee, J.C. et al. (1994). A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature, 372, 739-46.

[46] Anderson, D.R. et al. (2005). Aminocyanopyridine inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2). Bioorg Med Chem Let, 15, 1587-90.

[47] Wu, J.P. et al. (2007). The discovery of carboline analogs as potent MAPKAP-K2 inhibitors. Bioorg Med Chem Lett, 17, 4664-9.

[48] Goldberg, D.R. et al. (2008). Pyrazinoindolone inhibitors of MAPKAP-K2. Bioorg Med Chem Lett, 18, 938-41.

[49] Velcicky, J. et al. Novel 3-aminopyrazole inhibitors of MK-2 discovered by scaffold hopping strategy. Bioorg Med Chem Lett, 20, 1293-7.

[50] Trujillo, J.I. et al. (2007). Novel tetrahydro-beta-carboline-1-carboxylic acids as inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2). Bioorg Med Chem Lett, 17, 4657-63.

[51] Chico, L.K., Van Eldik, L.J. and Watterson, D.M. (2009). Targeting protein kinases in central nervous system disorders. Nat Rev Drug Discov, 8, 892-909.

[52] Arthur, J.S. (2008). MSK activation and physiological roles. Front Biosci, 13, 5866-79.

[53] Wiggin, G.R., Soloaga, A., Foster, J.M., Murray-Tait, V., Cohen, P. and Arthur, J.S. (2002). MSK1 and MSK2 are required for the mitogen- and stress-induced phosphorylation of CREB and ATF1 in fibroblasts. Mol Cell Biol, 22, 2871-81.

[54] Ananieva, O. et al. (2008). The kinases MSK1 and MSK2 act as negative regulators of Toll-like receptor signaling. Nat Immunol, 9, 1028-36.

[55] Darragh, J., Ananieva, O., Courtney, A., Elcombe, S. and Arthur, J.S. MSK1 regulates the transcription of IL-1ra in response to TLR activation in macrophages. Biochem J, 425, 595-602.

[56] Webber, K.M., Smith, M.A., Lee, H.G., Harris, P.L., Moreira, P., Perry, G. and Zhu, X. (2005). Mitogen- and stress-activated protein kinase 1: convergence of the ERK and p38 pathways in Alzheimer's disease. J Neurosci Res, 79, 554-60.

[57] Mosser, D.M. and Zhang, X. (2008). Interleukin-10: new perspectives on an old cytokine. Immunol Rev, 226, 205-18.

[58] Gaestel, M., Kotlyarov, A. and Kracht, M. (2009). Targeting innate immunity protein kinase signalling in inflammation. Nat Rev Drug Discov 8, 480-99.

[59] Duraisamy, S., Bajpai, M., Bughani, U., Dastidar, S.G., Ray, A. and Chopra, P. (2008). MK2: a novel molecular target for anti-inflammatory therapy. Expert Opin Ther Targets, 12, 921-36.

[60] Jeffrey, K.L., Camps, M., Rommel, C. and Mackay, C.R. (2007).Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. Nat Rev Drug Discov, 6, 391-403.

[61] Kim, S.H., Smith, C.J. and Van Eldik, L.J. (2004). Importance of MAPK pathways for microglial pro-inflammatory cytokine IL-1 beta production. Neurobiol Aging 25, 431-9.

[62] Koistinaho, M. and Koistinaho, J. (2002). Role of p38 and p44/42 mitogen-activated protein kinases in microglia. Glia, 40, 175-83.

[63] McDonald, D.R., Bamberger, M.E., Combs, C.K. and Landreth, G.E. (1998). beta-Amyloid fibrils activate parallel mitogen-activated protein kinase pathways in microglia and THP1 monocytes. J Neurosci, 18, 4451-60.

[64] Ferrer, I. (2004). Stress kinases involved in tau phosphorylation in Alzheimer's disease, tauopathies and APP transgenic mice. Neurotox Res, 6, 469-75.

[65] Giovannini, M.G., Scali, C., Prosperi, C., Bellucci, A., Vannucchi, M.G., Rosi, S., Pepeu, G. and Casamenti, F. (2002). Beta-amyloid-induced inflammation and cholinergic hypofunction in the rat brain in vivo: involvement of the p38MAPK pathway. Neurobiol Dis, 11, 257-74.

[66] Savage, M.J., Lin, Y.G., Ciallella, J.R., Flood, D.G. and Scott, R.W. (2002). Activation of c-Jun N-terminal kinase and p38 in an Alzheimer's disease model is associated with amyloid deposition. J Neurosci, 22, 3376-85.

[67] Takuma, K. et al. (2009). RAGE-mediated signaling contributes to intraneuronal transport of amyloid-beta and neuronal dysfunction. Proc Natl Acad Sci U S A, 106, 20021-6.

[68] Origlia, N. et al. (2008). Receptor for advanced glycation end product-dependent activation of p38 mitogen-activated protein kinase contributes to amyloid-beta-mediated cortical synaptic dysfunction. J Neurosci, 28, 3521-30.

[69] Zhu, X., Mei, M., Lee, H.G., Wang, Y., Han, J., Perry, G. and Smith, M.A. (2005). P38 activation mediates amyloid-beta cytotoxicity. Neurochem Res, 30, 791-6.
[70] Morfini, G.A. et al. (2009). Axonal transport defects in neurodegenerative diseases. J Neurosci 29, 12776-86.
[71] Cuenda, A. and Rousseau, S. (2007). p38 MAP-kinases pathway regulation, function and role in human diseases. Biochim Biophys Acta, 1773, 1358-75.
[72] Ferrer, I., Gomez-Isla, T., Puig, B., Freixes, M., Ribe, E., Dalfo, E. and Avila, J. (2005). Current advances on different kinases involved in tau phosphorylation, and implications in Alzheimer's disease and tauopathies. Curr Alzheimer Res, 2, 3-18.
[73] Peel, A.L., Sorscher, N., Kim, J.Y., Galvan, V., Chen, S. and Bredesen, D.E. (2004). Tau phosphorylation in Alzheimer's disease: potential involvement of an APP-MAP kinase complex. Neuromolecular Med, 5, 205-18.
[74] Dalrymple, S.A. (2002). p38 mitogen activated protein kinase as a therapeutic target for Alzheimer's disease. J Mol Neurosci, 19, 295-9.
[75] Anderton, B.H. et al. (2001). Sites of phosphorylation in tau and factors affecting their regulation. Biochem Soc Symp, 73-80.
[76] Li, Y., Liu, L., Barger, S.W. and Griffin, W.S. (2003). Interleukin-1 mediates pathological effects of microglia on tau phosphorylation and on synaptophysin synthesis in cortical neurons through a p38-MAPK pathway. J Neurosci, 23, 1605-11.
[77] Sun, A., Liu, M., Nguyen, X.V. and Bing, G. (2003). P38 MAP kinase is activated at early stages in Alzheimer's disease brain. Exp Neurol ,183, 394-405.
[78] Pei, J.J., Braak, E., Braak, H., Grundke-Iqbal, I., Iqbal, K., Winblad, B. and Cowburn, R.F. (2001). Localization of active forms of C-jun kinase (JNK) and p38 kinase in Alzheimer's disease brains at different stages of neurofibrillary degeneration. J Alzheimers Dis, 3, 41-48.
[79] Zhu, X. et al. (2001). Activation of MKK6, an upstream activator of p38, in Alzheimer's disease. J Neurochem, 79, 311-8.
[80] Zhu, X., Rottkamp, C.A., Boux, H., Takeda, A., Perry, G. and Smith, M.A. (2000). Activation of p38 kinase links tau phosphorylation, oxidative stress, and cell cycle-related events in Alzheimer disease. J Neuropathol Exp Neurol, 59, 880-8.
[81] Hensley, K. et al. (1999). p38 kinase is activated in the Alzheimer's disease brain. J Neurochem, 72, 2053-8.